REMARKS

The Examiner has rejected claims 1-6, 11, and 14 under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 5,993,844 (the '844 patent). The Examiner indicates that the '844 patent anticipates claims 1-6, 11, and 14 because the '844 patent describes graft constructs that are endotoxin-free and, according to the Examiner, a graft construct that is "endotoxin-free" is within the ranges claimed in the present application. Applicants respectfully traverse the Examiner's rejection. Claims 1-6, 11, and 14 are not anticipated by the '844 patent.

Anticipation exists only if all the elements of the claimed invention are present in a product or process disclosed, expressly or inherently, in a single prior art reference. *Hazeltine Corp. v. RCA Corp.*, 468 U.S. 1228 (1984). The '844 patent describes a graft prosthesis from which non-collagenous material has been removed (see column 4, lines 1-3 of the '844 patent). The graft construct described in the '844 patent is rendered substantially free of glycoproteins, glycosaminoglycans, proteoglycans, and non-collagenous proteins (see column 3, lines 41-44). The graft constructs claimed in claims 1-6, 11, and 14 of the present application comprise a "collagen-based matrix structure." As stated on page 14, lines 1-2 of the present application, the matrices in accordance with the present invention contain one or more naturally occurring components including glycoproteins, glycosaminoglycans, and proteoglycans and/or growth factors. The '844 patent describes graft prostheses from which these components have been removed. Thus, the '844 patent cannot anticipate claims 1-6, 11, and 14. Withdrawal of the rejection of claims 1-6, 11, and 14 under 35 U.S.C. § 102(e) as being anticipated by the '844 patent is respectfully requested.

Moreover, the Examiner contends that U.S. Provisional Application Serial No. 60/024,542 does not contain support for the phrase "5 endotoxin units per gram" in claim 3 because, although the '542 application recites less than 5 "EU/g," the '542 application does not set forth the meaning of "EU/g" and, according to the Examiner, this phrase cannot be assumed to mean "endotoxin units per gram." Contrary to the Examiners contention, it was well-known before the priority date of the present application that "EU/g" means "endotoxin units per gram."

Applicants transmit herewith Exhibit A, a paper authored by Dutkiewicz et al. and published in 1992 (Dutkiewicz et al., *Internatl. Biodet. & Biodeg.* Vol. 30: 29-46 (1992)). The passages bracketed on pages 32 and 33 of Dutkiewicz et al. show that it was well-known before the priority date of the present application that "EU/g" means "endotoxin units per gram." Because it was well-known before the priority date of the present application that "EU/g" means "endotoxin units per gram," the '542 application provides support for the phrase "less than 5 endotoxin units per gram" in claim 3. Thus, the '844 patent is not proper prior art to claim 3. Applicants further request withdrawal of the rejection of claim 3 under 35 U.S.C. § 102(e) as being anticipated by the '844 patent because the '844 patent is not proper prior art to claim 3.

The Examiner has rejected claims 7-10 and 16-20 under 35 U.S.C. § 102(e) as allegedly being anticipated by the '844 patent or, in the alternative, as being obvious under 35 U.S.C. § 103(a) over the '844 patent. The arguments discussed above, in the first two paragraphs of this section of the response, apply with equal force to this rejection. Moreover, the subject matter of claims 9, 10, and 16-20 is not disclosed or suggested by the '844 patent. The '844 patent provides no suggestion of cleaning the graft prosthesis prior to delamination (see claims 9, 10, and 16-20). Withdrawal of the rejection of claims 7-10 and 16-20 under 35 U.S.C. § 102(e) as being anticipated by the '844 patent, or under 35 U.S.C. § 103(a) as being obvious over the '844 patent is respectfully requested, based on both of the foregoing arguments.

The Examiner has rejected claims 12 and 13 under 35 U.S.C. § 103(a) as being obvious over the '844 patent. The Examiner has also rejected claim 15 under 35 U.S.C. § 103(a) as being obvious over the '844 patent in combination with Braun. The arguments discussed above, in the first two paragraphs of this section of the response, apply with equal force to this rejection in the context of obviousness. Withdrawal of the rejection of claims 12 and 13 under 35 U.S.C. § 103(a) as being obvious over the '844 patent is respectfully requested. Withdrawal of the rejection of claim 15 under 35 U.S.C. § 103(a) as being obvious over the '844 patent in combination with Braun is respectfully requested.

The Examiner has requested that Applicants provide a list of all copending

applications that set forth subject matter similar to the claims of the above-captioned application, as well as a copy of the co-pending claims. Such applications are co-pending U.S. Patent Application Nos. 10/744,420 and 10/811,343, the claims of which were submitted as Appendix A and Appendix B, respectively, to the response filed on December 3, 2004 in this application.

The Examiner also pointed in the office action to Rule 41.202, in particular sections (a)(4) and (d), covering the requirement to make a showing of priority. The present application claims the same priority date as the patent or published application claiming interfering subject matter so it is Applicants' understanding that a priority showing under Rule 41.202(a)(4) and (d) is not required for the present application.

CONCLUSION

The foregoing remarks are believed to fully respond to the Examiner's rejections. Applicants respectfully request issuance of an action indicating that the claims are allowable, and issuance of a declaration of interference.

Respectfully submitted,

Rebecca L. Ball
Registration No. 46,535
Attorney for Applicants

RVB:wlb
Indianapolis, Indiana 46204
317-231-7511
INDS02 RVB 790548v1

Exhibit A

International Biodeterioration & Biodegradation **30** (1992) 29–46

Levels of Bacteria, Fungi and Endotoxin in Stored Timber*

Jacek Dutkiewicz,[a,b] W. G. Sorenson,[a] Daniel M. Lewis,[a] & Stephen A. Olenchock[a †]

[a] Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health (NIOSH), Morgantown, West Virginia 26505, USA
[b] Institute of Agricultural Medicine, Lublin, PL-20-124 Poland

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ABSTRACT

Four series of wood samples were taken from the heartwood, sapwood and bark of six species of timber logs (American basswood, black cherry, black locust, red oak, soft maple and white poplar) in the summer, fall, winter and spring. The samples were examined by dilution plating for total aerobic bacteria, gram-negative bacteria and fungi. The chromogenic modification of the Limulus *amebocyte lysate test was also used for bacterial endotoxin. The numbers of the micro-organisms and endotoxin in the wood were significantly higher during warm periods (spring and summer) than during cold periods (fall and winter). They were highest in the wood of American basswood and black locust, exceeding the levels of* 10^7 *cfu* g^{-1} *and* 10^5 *endotoxin units* g^{-1}*, respectively. Gram-negative bacteria and* Corynbacterium *spp. prevailed among aerobic bacteria recovered from heartwood and sapwood, while* Bacilli *spp. were the most common in the bark.* Enterobacter agglomerans *(synonym:* Erwinia herbicola*),* Agrobacterium radiobacter, Pseudomonas fluorescens, Pseudomonas maltophilia & Acinetobacter calcoaceticus *were most common among gram-negative bacteria. Yeasts dominated the*

† To whom correspondence should be addressed at: Immunology Section, NIOSH, 944 Chestnut Ridge Road, Morgantown, West Virginia 26505.

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fungal flora of heartwood and sapwood, whereas filamentous fungi constituted a prevailing fraction in the bark. The results indicate that the microflora of the timber that apparently was undecayed may reach high levels and may contain allergenic and/or toxic species which pose a potential risk for sawmill workers.

INTRODUCTION

Stored timber is often colonized by bacteria (Liese & Karnop, 1968; Greaves, 1971; Rossell *et al.*, 1973) and fungi (Greaves & Levy, 1968; Käärik, 1975; Levy, 1975). Early colonization is characterized by development of different types of bacteria (Greaves, 1971; Rossell *et al.*, 1973). Gram negative bacteria have also been reported from tissues of living trees, mainly from those described as 'wetwood' (Shigo & Hills, 1973; Murdoch & Campana, 1983; Jagels, 1985). Bacteria may affect the permeability of wood and cause progressive damage to the wood structure (Greaves, 1971). Their presence could be accompanied by the presence of fungi, mainly yeasts (Greaves & Levy, 1968; Shigo & Hills, 1973; Käärik, 1975; Levy, 1975). The second, advanced stage of microbial colonization of timber is characterized by abundant growth of wood-rotting hymenomycetous fungi, leading to rapid decay of wood tissue (Käärik, 1975; Levy, 1975).

Both synergistic and antagonistic effects of bacteria and fungi in wood have been reported (Greaves & Levy, 1968; Rossell *et al.*, 1973; Levy, 1975), but little is known about the quantitative proportions of different organisms in various kinds of wood. This information could be useful not only from the viewpoint of wood technology, but also from the viewpoint of the health protection of the workers who are exposed to inhaled wood dust contaminated with micro-organisms and their toxic and/or allergenic products. There is growing evidence that exposure to wood dust when working in a sawmill or handling wood chips may result in pulmonary disease, such as hypersensitivity pneumonitis (allergic alveolitis), asthma, organic dust toxic syndrome (ODTS) and chronic bronchitis (Terho *et al.*, 1980; Wimander & Belin, 1980; Rosenhall *et al.*, 1982; Jagels, 1985; Asmussen *et al.*, 1986; Kolmodin-Hedman *et al.*, 1987).

Most of the reports on adverse effects of the wood-borne micro-organisms on the human respiratory system refer to fungi belonging to the following species and/or genera: *Alternaria tenuis* (Rylander & Goto, 1989), *Aspergillus fumigatus* (Minarik *et al.*, 1983; Sneath *et al.*, 1986; Land *et al.*, 1987), *Aureobasidium pullulans* and *Graphium* (Cohen *et al.*, 1967), *Cryptostroma corticale* (Emanuel *et al.*, 1966), *Paecilomyces, Mucor,* and *Rhizopus* (Wilhelmsson *et al.*, 1984; van Assendelft *et al.*, 1985), *Penicillium*

(Avila & Lacey, 1974; Terho *et al.*, 1980; Dykewicz *et al.*, 1988) and *Trichoderma* and *Scopulariopsis* (Halprin *et al.*, 1973). Thermophilic actinomycetes from the species *Saccharomonospora viridis* and *Thermoactinomyces vulgaris* (Greene *et al.*, 1981; Sneath *et al.*, 1986), *Bacillus subtilis* (Johnson *et al.*, 1980), and the endotoxin-producing gram-negative bacteria from the genus *Enterobacter* have been suggested as potential disease factors (Jagels, 1985; Dutkiewicz, 1989).

The purpose of this study was to extend the knowledge of the potential respiratory risk to woodworkers from wood-inhabiting micro-organisms in timber scheduled for processing in a sawmill. The study was designed as a season-oriented, quantitative and qualitative survey of the types of micro-organisms in different tree species which might be aerosolized during processing of the timber.

MATERIALS AND METHODS

Four series of wood samples were obtained during August and October of 1987, and February and May of 1988, from logs stored on the lumber yard at a sawmill in Kingwood, West Virginia. The air temperatures on sampling days were 26·5 °C, 5·5 °C, −4 °C and 20·5 °C, respectively. The logs had been stored before sampling for a period of 4–6 weeks and did not show any visible signs of decay. At each sampling time, samples were taken randomly from the cross-cut end of a log of each of the following species: American basswood (*Tilia americana* L.), black cherry (*Prunus serotina Ehrh.*), black locust (*Robinia pseudoacacia* L.), red oak (*Quercus coccinea* Muench.), soft maple (*Acer saccharinum* L.) and white poplar (*Populus alba* L.). From each log, one sample was taken from the heartwood (by boring from the transverse section), one from the sapwood (by boring from the transverse section) and one from the bark (by centripetal boring).

The wood samples were collected with a novel 'drill and collect' device (model #2) for quantification of micro-organisms in wood (Dutkiewicz *et al.*, 1989). This is a manually operated drilling device in which the combined action of a twist boring bit and a spring-containing mobile ring collects the pulverized wood into a sterile flask attached beneath the bit in a one-step process. The wood surface to be sampled was first disinfected by wiping with 70% propanol and 'Clorox' (a commercial 5·25% sodium hypochlorite solution) and then an average sample was taken by multiple boring (5–7 times) in a circle up to 3 cm in diameter.

The numbers of bacteria and fungi in the wood samples were determined by dilution plating. Aliquots of 200 mg of each sample were

suspended in 20 ml of sterile phosphate buffered saline (Sigma Chemical Co., St Louis, MO) containing 0·1% (v/v) Tween 80 (Fisher Scientific Co., Fair Lawn, NJ) and, after vigorous shaking, serial 10-fold dilutions were made up to 10^{-6}. The 0·1 ml aliquots of each dilution were spread on duplicate sets of the following agar media: (i) sheep blood agar for total aerobic bacteria (mesophilic), (ii) eosin methylene blue agar (EMB agar; DIFCO Lab., Detroit, MI) for gram-negative bacteria, (iii) half-strength tryptic soya agar (DIFCO) for thermophilic actinomycetes, (iv) rose bengal streptomycin agar (RBS) (Rogerson, 1958) for filamentous fungi and (v) yeast malt agar (Kreger-van Rij, 1984) for yeasts. The blood agar and EMB plates were incubated for 48 h at 35 °C, the tryptic soya agar plates for 120 h at 55 °C and the RBS and yeast malt agar plates for 96 h at 28 °C.

Following incubation, bacterial colonies were counted and differentiated on the basis of colony morphology, Gram reaction and biochemical reactions. The Gram-negative isolates were identified with the API Systems 20 E (for enterobacteria) and NFT (for non-fermenting bacteria) (API Analytab Products, Plainview, NY), using supplementary biochemical tests selected according to Bergey's Manual (Krieg & Holt, 1984), and API Systems recommendations. The gram-positive isolates were identified according to Bergey's Manual (Sneath *et al.*, 1986), using API Systems 20 S for identification of streptococci and STAPH Trac for identification of staphylococci. Colonies of filamentous fungi were counted and differentiated on the basis of morphological properties. Representative yeast colonies were isolated and differentiated on the basis of morphological and biochemical properties (Kreger-van Rij, 1984). Final results for microbial numbers were reported in terms of the colony-forming units (cfu) per gram of pulverized wood.

For endotoxin determination, 100 mg portions of the wood samples were extracted with 5 ml of sterile non-pyrogenic water (Travenol Laboratories Inc., Deerfield, IL) by rocking for 60 min at room temperature. The suspension was centrifuged at 1000 g for 10 min to remove particulate debris, and the supernatant fluid was separated for further analysis. Quantification of gram-negative bacterial endotoxin content was performed in duplicate by a quantitative chromogenic modification of the *Limulus* amebocyte lysate test (QCL-1000; Whittaker Bioproducts, Walkersville, MA). Results were reported in terms of endotoxin units (EU) per gram of pulverized wood.

The Wilcoxon test for matched pairs and the test for multiple regression were used for statistical analysis of the results (with the aid of the STATS+/Statsoft software package). The software packages Sigma-Plot (Jandel Scientific, Corte Madera, CA) and Harvard Graphics

(Software Publishing Corp., Mountain View, CA) were used for graphic presentation of the results.

RESULTS

Numbers of microorganisms and endotoxin in wood

The numbers of total aerobic bacteria, Gram-negative bacteria and fungi ranged up to 10^8 cfu/g of wood (Figs 1–3) and concentration of endotoxin was in the range of 10^0–10^6 EU/g of wood (Fig. 4). No thermophilic actinomycetes were found in the wood samples examined.

The numbers of micro-organisms and endotoxin concentration in wood were higher during warm periods (spring, summer) than during cold periods (fall, winter). These differences were more distinct in the heartwood and sapwood than in bark. No Gram-negative bacteria and fungi could be recovered from the heartwood of any log species during the winter sampling (Figs 2–3). Spring levels of total bacteria, Gram-negative bacteria, fungi and endotoxin were significantly higher than fall and winter levels ($P < 0{\cdot}05$). Similarly, the summer level of fungi was significantly higher than fall and winter levels ($P < 0{\cdot}05$) and the summer level of Gram-negative bacteria was significantly higher than the winter level ($P < 0{\cdot}05$). In general, the levels of Gram-negative bacteria and of Corynebacteria were highest in warm periods (spring and summer), whereas the levels of Bacilli were highest during cold periods (fall and winter).

The levels of micro-organisms and endotoxin in wood samples varied significantly with the species of log examined. In most cases they were high ($>10^4$ cfu/g or $>10^3$ EU/g) in samples from American basswood and black locust while in the samples from the remaining four species the levels were either low or variable. This was especially true of total bacterial counts which were higher in the samples from basswood and locust than in the samples from cherry, maple, oak and polar ($P < 0{\cdot}05$).

The levels of Gram-negative bacteria and of endotoxin were significantly higher in the wood of basswood and locust than in the wood of cherry, maple and oak ($P < 0{\cdot}05$). The level of fungi in the wood of basswood was significantly higher than in the wood of cherry, oak and poplar ($P < 0{\cdot}05$) and the same level in locust was significantly higher than in cherry ($P < 0{\cdot}01$).

The counts of total bacteria and fungi in bark of logs studied were significantly greater than in the heartwood ($P < 0{\cdot}02$). They were also higher than in the sapwood, but the difference did not prove to be

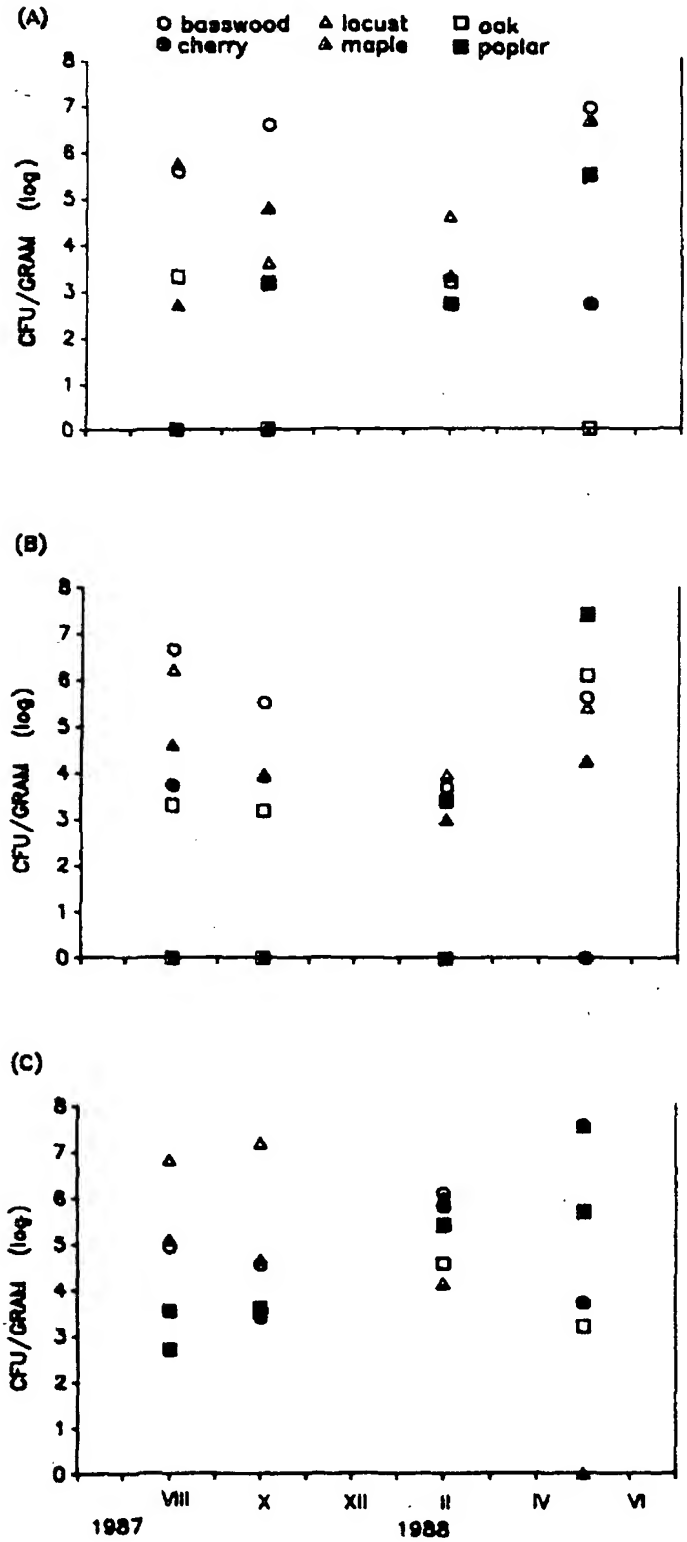

Fig. 1. The numbers of total aerobic bacteria in the heartwood (A), sapwood (B) and bark (C) of six different species of logs throughout four seasons.

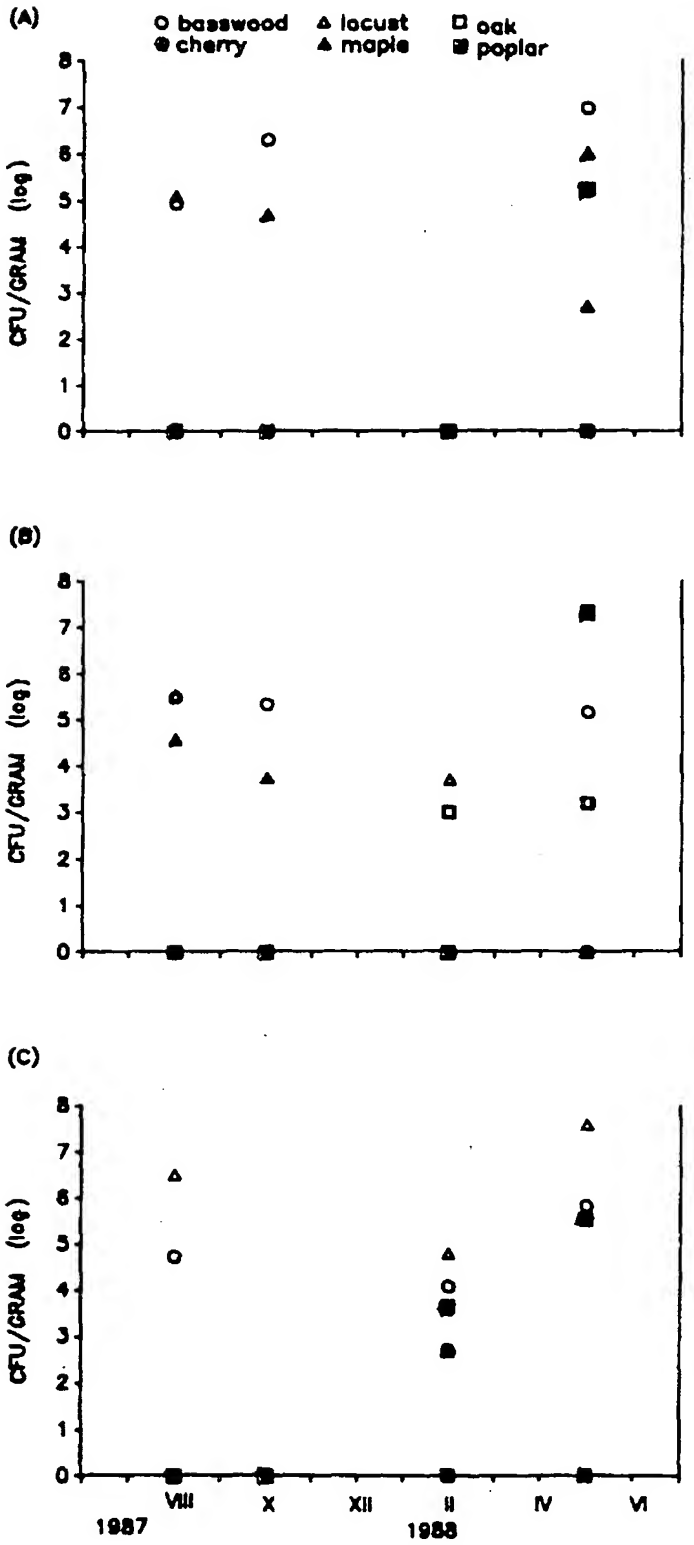

Fig. 2. The numbers of gram-negative bacteria in the heartwood (A), sapwood (B) and bark (C) of six different species of logs throughout four seasons.

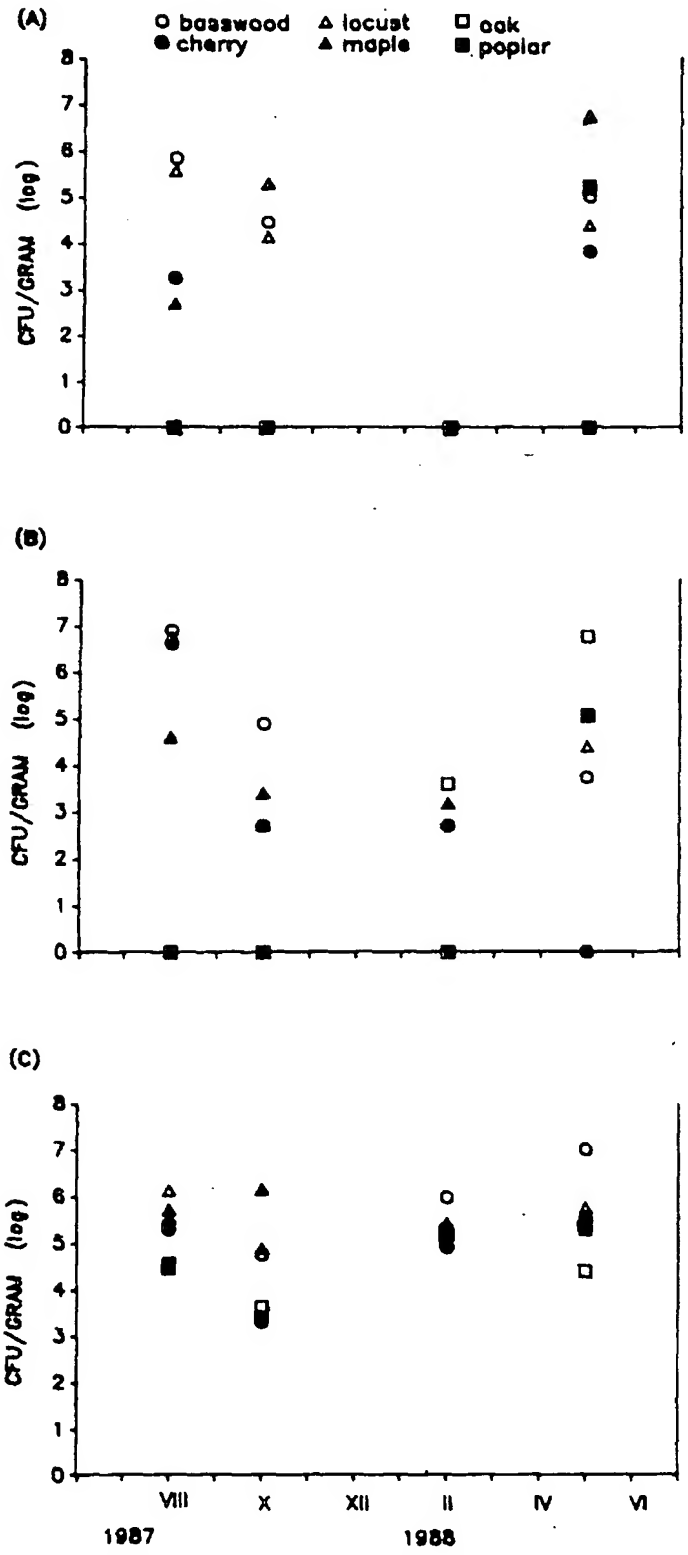

Fig. 3. The numbers of fungi in the heartwood (A), sapwood (B) and bark (C) of six different species of logs throughout four seasons.

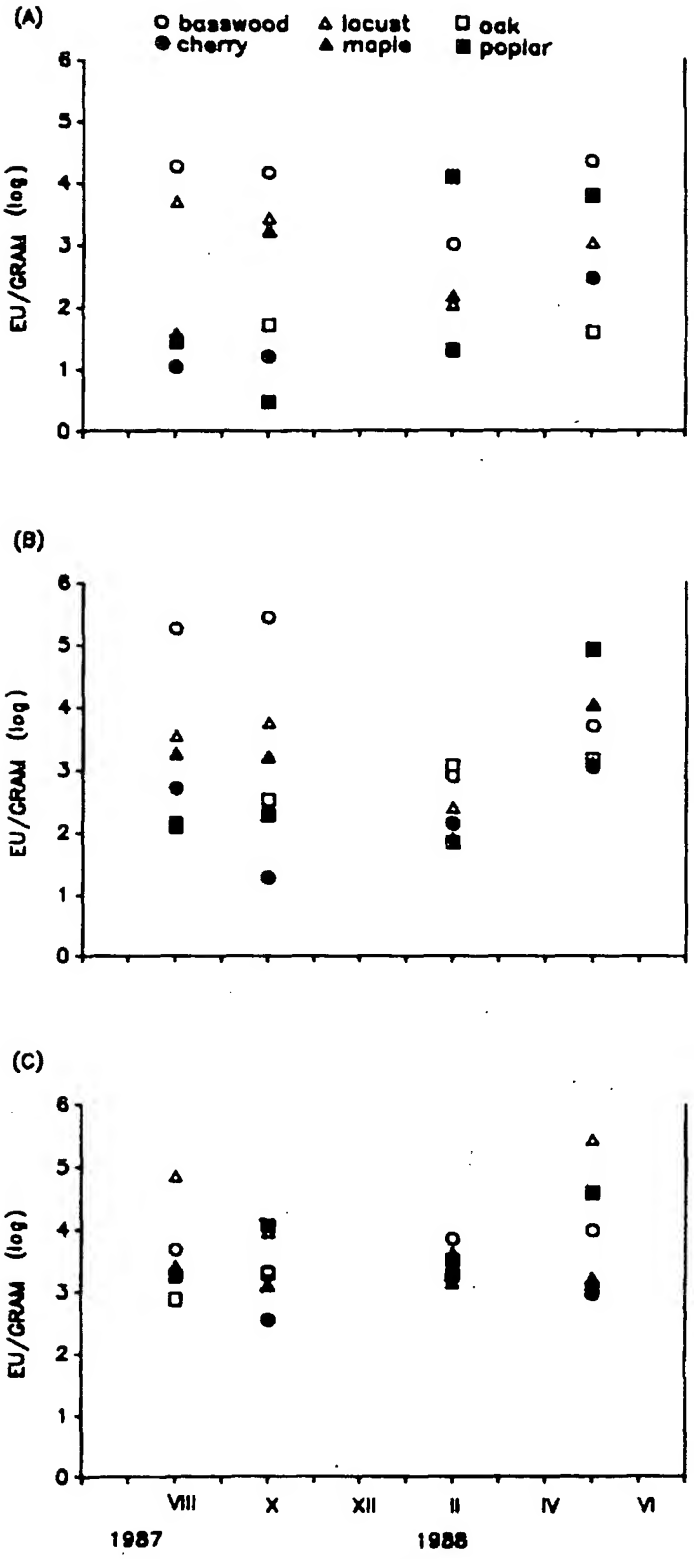

Fig. 4. The concentration of endotoxin in the heartwood (A), sapwood (B) and bark (C) of six different species of logs throughout four seasons.

significant ($0{\cdot}05 < P < 0{\cdot}1$). No significant differences could be found between the number of Gram-negative bacteria in the heartwood, sapwood and bark ($P > 0{\cdot}7$). The level of endotoxin in the sapwood was significantly higher than in the heartwood ($P < 0{\cdot}05$), but no significant differences were found between the endotoxin level in bark and in heartwood and sapwood ($0{\cdot}05 < P < 0{\cdot}1$).

Correlation between the levels of particular microorganisms and endotoxin

The levels of total bacteria were significantly correlated with the levels of fungi ($r = 0{\cdot}413$, $P < 0{\cdot}001$) and of endotoxin ($r = 0{\cdot}457$, $P < 0{\cdot}001$). No correlation could be found between the levels of fungi and of Gram-negative bacteria and endotoxin ($P > 0{\cdot}1$).

A highly significant correlation was found between the levels of Gram-negative bacteria and endotoxin (Fig. 5). However, this correlation was not consistent for all kinds of wood. It was highly significant for the heartwood and bark samples ($P < 0{\cdot}0001$), but not for the sapwood samples ($P > 0{\cdot}3$). This correlation had the highest level of significance for the locust and poplar samples ($P < 0{\cdot}0001$), lower significance for the cherry samples ($P < 0{\cdot}01$) and was not significant for the basswood, maple and oak samples ($P > 0{\cdot}2$).

Composition of the microflora

The genera and/or species of bacteria and fungi found in the wood samples are listed in Table 1. A number of non-sporulating fungi could not be identified.

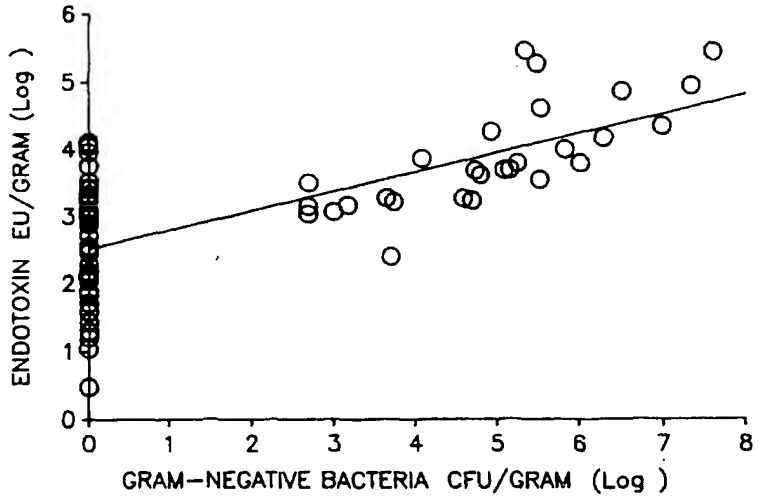

Fig. 5. The regression line showing a correlation between the numbers of gram-negative bacteria and amount of endotoxin for the total of wood samples.

TABLE 1
Species and/or Genera of Bacteria and Fungi in Wood Sampies

Gram-negative bacteria:
- *Acinetobacter calcoaceticus* var. *anitratus*
- *Acinetobacter calcoaceticus* var. *lwoffi*
- *Agrobacterium radiobacter*
- *Alcaligenes faecalis*
- *Citrobacter diversus*
- *Citrobacter freundii*
- *Enterobacter agglomerans* (Synonym: *Erwinia herbicola*)
- *Enterobacter cloacae*
- *Klebsiella* spp.
- *Pseudomonas fluorescens*
- *Pseudomonas maltophilia*
- *Pseudomonas oryzihabitans* (synonym: CDC gr. V E-2)
- *Pseudomonas paucimobilis*
- *Pseudomonas putida*
- *Pseudomonas stutzeri*
- *Pseudomonas vesicularis*
- *Serratia rubidaea*

Gram-positive bacteria:
- *Arthrobacter* spp.
- *Bacillus subtilis*
- *Bacillus* spp.
- *Brevibacterium linens*
- *Corynebacterium* spp.
- *Microbacterium* spp.
- *Micrococcus luteus*
- *Rhodococcus* sp.
- *Staphylococcus sciuri*
- *Staphylococcus simulans*
- *Staphylococcus* spp.
- *Streptococcus* spp.
- *Streptomyces* spp.

Yeasts:
- *Candida* spp.
- *Cryptococcus* spp.
- *Hansenula* spp.
- *Rhodotorula* spp.

Filamentous fungi:
- *Acremonium (Cephalosporium)* sp.
- *Alternaria* sp.
- *Aspergillus fumigatus*
- *Aureobasidium pullulans*
- *Bispora* sp.
- *Cladosporium* sp.
- *Mortierella* sp.
- *Oidiodendron* sp.
- *Penicillium* sp.
- *Pestalotia* sp.
- *Phoma* sp.
- *Trichoderma* sp.
- *Umbelopsis* sp.
- *Verticillium* sp.
- non-sporulating fungi

The average data on the percentage composition of the microflora of wood are presented in Figs 6–8. Among the total aerobic bacteria (Fig. 6), the relatively most common organisms were gram-negative bacteria. These organisms prevailed in half of the heartwood or sapwood samples and in one third of the bark samples, being particularly common in the wood of basswood and poplar. The next most common organisms were Bacilli which were dominant in the bark in three of six cases, and Corynebacteria which prevailed in the heartwood and sapwood in four of six cases. Streptococci proved to be dominant once in the sapwood of oak and once in the bark of basswood, and staphylococci were predominant once in the heartwood of oak.

Figure 7 shows a notable diversity of the Gram-negative flora of the woods examined. The species most commonly found in the wood were *Enterobacter agglomerans, Agrobacterium radiobacter, Pseudomonas maltophilia* and *Pseudomonas fluorescens*, whereas in the bark the most

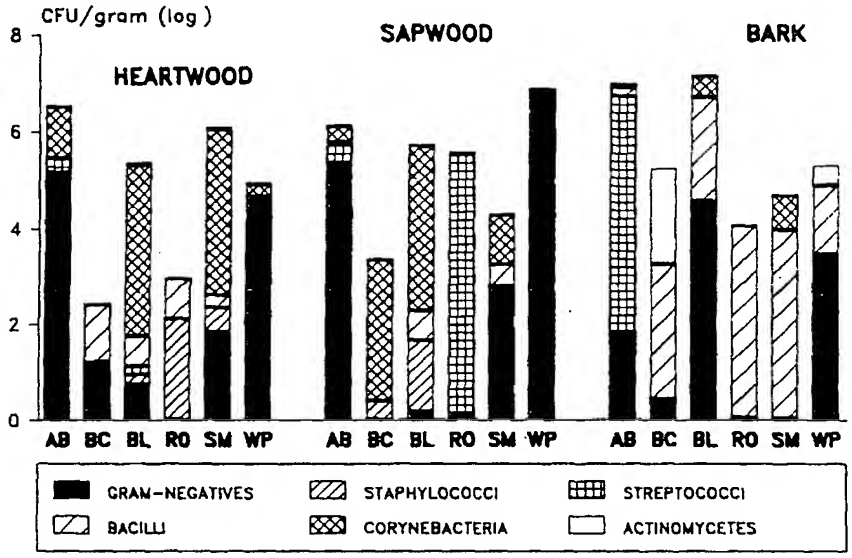

Fig. 6. The composition of the aerobic bacterial flora in the heartwood, sapwood, and bark of six different species of logs (average data for all seasons). AB—American basswood; BC—black cherry; BL—black locust; RO—red oak; SM—soft maple; WP—white poplar.

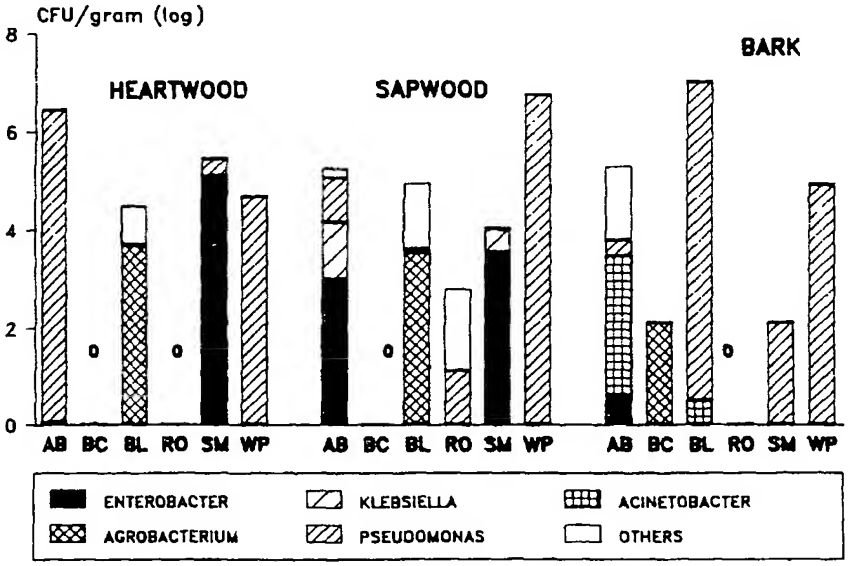

Fig. 7. The composition of the gram-negative flora in the heartwood, sapwood, and bark of six different species of logs (average data for all seasons). 0 = no gram-negative bacteria found. Abbreviations are the same as in Fig. 6.

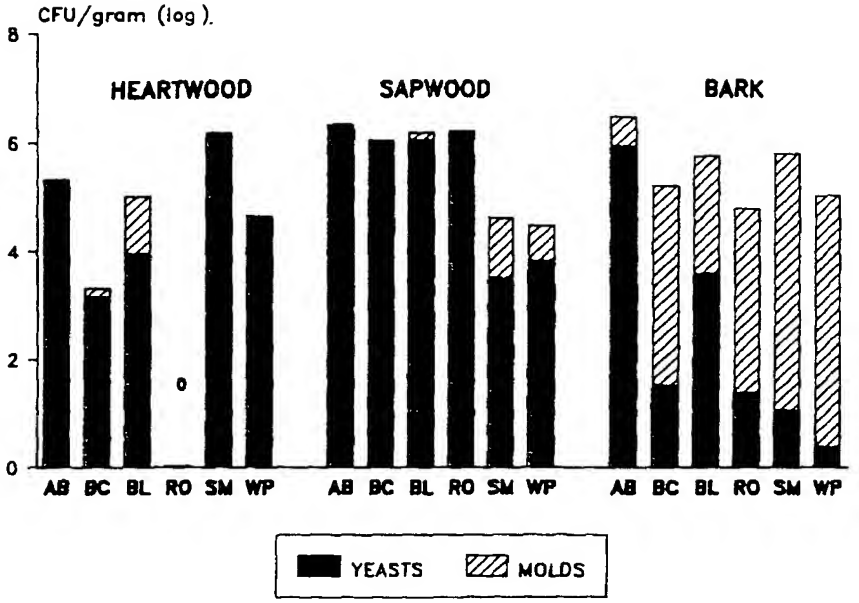

Fig. 8. The composition of the fungal flora in the heartwood, sapwood, and bark of six different species of logs (data cumulated for all seasons). 0 = no fungi found. Abbreviations are the same as in Fig. 6.

common were *Pseudomonas fluorescens* and *Acinetobacter calcoaceticus*. High percentages of *Ps. fluorescens* and *Ps. maltophilia* in the total Gram-negative flora are mainly due to very high levels of these bacteria found in the samples taken in spring 1988.

Yeasts dominated the heartwood and sapwood of all the log species examined (Fig. 8). The most numerous among yeasts were presumptive Ascomycetes and their anamorphs, i.e. organisms which gave a negative reaction with Diazonium Blue B (DBB) (Kreger-van Rij, 1984). The level of filamentous fungi in the heartwood and sapwood was very low and in most cases did not exceed 1% of the total fungal flora observed. Conversely, filamentous fungi prevailed among fungi found in the bark in four of the six tree species studied. The most common genera of filamentous fungi were *Acremonium, Oidiodendron, Phoma, Penicillium, Pestalotia, Trichoderma, Umbelopsis* and *Verticillium*.

DISCUSSION

This study extends the knowledge of the potential respiratory risk to woodworkers from wood-inhabiting micro-organisms which might be aerosolized during processing of the wood. The data presented confirm the views of earlier authors (Greaves & Levy, 1968; Rossell *et al.*, 1973) on

the diversity of the microflora of wood and on the possiblity of a positive correlation between bacterial and fungal populations. The composition of the microflora of logs studied, characterized by the prevalence of yeasts and Gram-negative bacteria suggests that it may have been in an early stage of colonization before the stage of wood decay by brown rot and white rot fungi (Shigo & Hills, 1973; Käärik, 1975). Many of the bacterial and fungal species or genera found in this study, including *E. agglomerans, E. cloacae, Klebsiella, A. faecalis, Acinetobacter, Ps. fluorescens, Streptococcus, Micrococcus, Staphylococcus, Bacillus, Corynebacterium, Penicillium, Aspergillus fumigatus* and *Aurobasidium pullulans* were reported by earlier investigators either from tissues of living trees (Basham & Taylor, 1965; Cosenza *et al.*, 1970; Murdoch & Campana, 1983; Dykewicz *et al.*, 1988) or from timber (Cohen *et al.*, 1967; Liese & Karnop, 1968; Greaves, 1971; Rossell *et al.*, 1973; Minarik *et al.*, 1983; van Assendelft *et al.*, 1985). There does not appear to be any previous report of *Agrobacterium radiobacter, Citrobacter diversus, Citrobacter freundii, Ps. Maltophilia, Ps. oryzihabitans, Ps. paucimobilis, Ps. putida, Ps. stutzeri* or *Ps. vesicularis* from wood.

The levels of micro-organisms and endotoxin in timber logs showed notable variation depending on the season, the species of the log and the kind of wood tissue. They were significantly higher during warm seasons (late spring and summer), suggesting the possibility of a greater degree of risk for sawmill workers from microbial aeroallergens and toxins during that period.

The numbers of bacteria and fungi in the wood of American basswood and black locust were significantly higher than in the wood of the remaining species examined (black cherry, red oak, soft maple, white poplar (Figs 6 and 8). This would suggest that these species (basswood and locust) might present a greater risk for microbial aerosolization than the other species. The levels of micro-organisms in the most contaminated wood samples were comparable to the values reported for certain organic dusts related to harmful respiratory effects in workers (Dutkiewicz, 1978). The risk could be in fact even greater if one considers a possibility of colonization of wood by anaerobic bacteria (Shigo & Hills, 1973) which were not determined in the present study.

The amount of endotoxin in the wood reached a level of 10^5–10^6 EU/g in many cases, which corresponds to the values found in organic materials (grain, silage, mushroom farm pre-flush) associated with the cases of respiratory disorders in exposed workers (Olenchock, 1988). This finding is in agreement with the fact that some of the wood samples contained high numbers of Gram-negative bacteria. Among these

bacteria were the species (*Enterobacter agglomerans, Klebsiella spp., Pseudomonas putida*) which are known producers of biologically active endotoxin that can cause pulmonary injury through non-specific stimulation of alveolar macrophages (Rylander & Snella, 1983). *Bacillus subtilis* has been shown to induce hypersensitivity (Johnson *et al.*, 1980) and its presence in the bark may be an additional risk factor.

The occurrence of high numbers of filamentous fungi in the bark of wood examined presents another factor of respiratory risk for sawmill workers, in particular for those engaged in debarking of logs or in processing of wastewood in paper mills, chipboards factories and similar facilities. The *Penicillium* species that were frequently isolated in this study have been reported as a source of pathogenic respiratory allergens (Avila & Lacey, 1974; van Assendelft *et al.*, 1985; Dykewicz *et al.*, 1988). Other potentially hazardous species are *Aureobasidium pullulans* (Cohen *et al.*, 1967), *Trichoderma sp.* (Halprin *et al.*, 1973) and *Aspergillus fumigatus* which is known as a cause of allergic alveolitis (Terho *et al.*, 1980; Minarik *et al.*, 1983) and as a producer of tremorgenic mycotoxin (Land *et al.*, 1987).

The significance of wood-borne yeasts as potential hazardous factors is less known and is the subject of a separate study (Sorenson *et al.*, 1991). In the light of the recent reports on the potential role of fungal glucans as inducers of a chronic pulmonary disease (Rylander & Goto, 1989), the possibility of both nonspecific and specific effects of yeasts must be considered.

In conclusion, our results indicate that some kinds of apparently undecayed timber logs stored for processing in sawmills may contain very high numbers of micro-organisms and their toxins. These organisms may potentially cause respiratory disorders in the woodworkers if inhaled with the sawdust during debarking and sawing operations. Our report indicates a need for further research of this problem and characterization of the microbial burden during various job activities within a sawmill.

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